Turk J Chem
28 (2004) , 441 – 449.

Synthesis and Microbiological Activity of Some Substituted N-(2−hydroxy-4-nitrophenyl)benzamides and Phenylacetamides as Possible Metabolites of Antimicrobial Active Benzoxazoles

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Received 11.11.2003

Some new antimicrobial active N-(2−hydroxy-4-nitrophenyl)-p-substituted benzamide and phenylacetamide analogues (**I-II**) were prepared by 2-step procedures from the corresponding carboxylic acids as possible metabolites of benzoxazoles. Their antimicrobial activities were tested against 3 Gram-positive and 2 Gram-negative bacteria with the fungus *Candida albicans* and were also compared with several control drugs. The compounds **Ia, Id, IIa**, and **IIe** were active at a MIC value of 12.5 μg/mL against the Gram-positive microorganism *Bacillus subtilis*. Most of the compounds exhibited antifungal activity at a MIC value of 12.5 μg/mL against *C. albicans*. On the other hand, the antimicrobial activity of some amide derivatives (**Ia, Id, IIa-c**) was also compared with their cyclic analogues, benzoxazole derivatives (**III-IV**). The compound **Id** significantly possessed 2 or 3 dilutions better antimicrobial activity than its heterocyclic derivative, 2-(p-*t*-butylphenyl)-5-nitrobenzoxazole, **IIId**, against *Staphylococcus aureus, Streptococcus faecalis, Klebsiella pneumonia*, and *Escherichia coli*.

Key Words: Antimicrobial activity, benzamide, benzoxazole, phenylacetamide.

Introduction

The number of life threatening infections caused by multidrug-resistant Gram-positive pathogens has reached an alarming level in hospitals and the community[1−3]. Infections caused by these organisms pose a serious challenge to the scientific community and the need for an effective therapy has led to a search for novel antibacterial agents.

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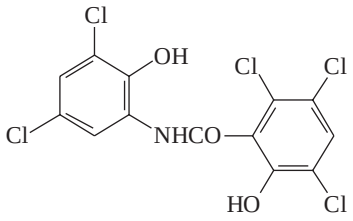
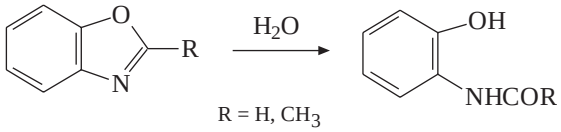
Benzoxazole derivatives constitute an important class of heterocyclic compounds for their antibacterial and antifungal activities[4−12]. Benzamide derivatives that are the possible metabolites of benzoxazoles exhibit various type of biological properties such as anthelminthic, antihistaminic, antifungal and antibacterial[13−18]. Oxyclozanide, which has a benzamide structure, was discovered in 1969 as an anthelmentic agent effective against *Fasciola hepatica* for the treatment of liver fluke infection[13]. 3,4-dihydroxy-6-(N-ethylamino)benzamide is a new natural product that has been found in green pepper (*Piper nigrum* L.) as an antibacterial by Variyar et al. in 1999[18]. However, there are few published data on the antibacterial and antifungal activity of the benzamide derivatives.



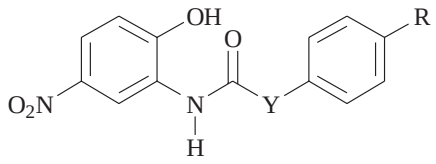
Oxyclozanide

A review of the literature revealed that one of the aspects of the Phase I metabolism pathways of benzoxazole in the rabbit involved cleavage of the oxazole ring at the (C-O) linkage of the fused heterocyclic system by mild hydrolysis and produced *o*-formamidophenol and *o*-acetamidophenol[19]. This is represented in Scheme 1, omitting the intermediate stages.



Scheme 1

In this study, we report the synthesis and the antimicrobiological activity of some new N-(2-hydroxy-4-nitrophenyl)benzamides (**Ia-d**) and phenylacetamides (**IIa-e**) (Figure 1) and their activity is compared to their cyclic analogues (**III-IV**) (Table 3), assuming that the amides are possible metabolites of these heterocyclic compounds.



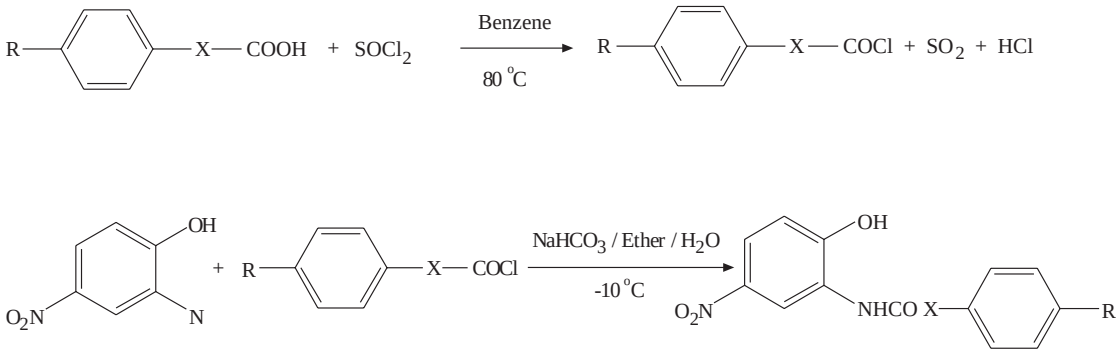
Y = —, CH_2

R = H, Cl, Br, NO_2, OCH_3, $C(CH_3)_3$, OC_3H_7

Figure 1

The synthesis of compounds **I-II** was performed by reacting suitable 2-aminophenols with appropriate carboxylic acid chlorides, obtained by treating carboxylic acids with thionyl chloride as given in Scheme 2.

The compounds **I-II** were prepared as new products, except for compounds **Ia**[20], **Ib**[21] and **IIc**[22]. All of the structures were supported by spectral data. The IR and ^{1}H NMR spectra are in agreement with the proposed structures. Physical and spectral data of the compounds are reported in Table 1.



Scheme 2

Experimental

Chemistry

Silicagel HF_{254} chromatoplates (0.3 mm) were used for TLC and the solvent systems were chloroform:methanol (20:1) for all compounds. All the melting points were taken on a Buchi SMP 20 capillary apparatus and are uncorrected. IR spectra were recorded by FT/IR - 420 with KBr discs. ^{1}H NMR spectra were obtained with a Bruker 400 MHz spectrometer in DMSO and d_6-chloroform and tetramethylsilane (TMS) was used as an internal standard. Elemental analyses were carried out with a Perkin Elmer model 240-C apparatus. The results of the elemental analyses (C, H, N) were within $\pm$ 0.4% of the calculated amounts.

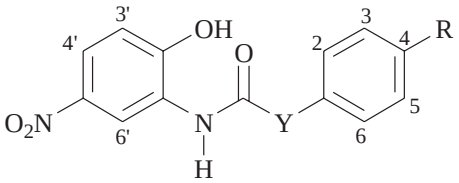
General procedure for the synthesis of N-(2-hydroxy-4-nitrophenyl)benzamides and phenylacetamides

Thionyl chloride (1.5 mL) and appropriate carboxylic acid (0.5 mmol) were refluxed in benzene (5 mL) at 80 °C for 3 h and then the excess thionyl chloride was removed *in vacuo*. The residue was dissolved in ether (10 mL) and the solution was added over 1 h to a stirred, ice-cold mixture of 2-amino-4-nitrophenol (0.5 mmol), sodium bicarbonate (0.5 mmol), diethyl ether (10 mL) and water (10 mL). The mixture was stirred overnight at room temperature and filtered. After the precipitate was washed with water, 2 N HCl, water and finally with ether **I-II** were obtained. An ethanol-water mixture was used for recrystallization and the crystals were dried *in vacuo*.

Microbiology

The compounds were dissolved in absolute ethanol (0.8 mg/mL) for both the antibacterial and antimycotic assays. Further dilutions of the compounds and standard drugs in the test medium were prepared at the required concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 μg/mL with Mueller-Hinton broth and Sabouraud dextrose broth. The minimum inhibitory concentrations (MIC) were determined using the 2-fold serial dilution technique[23,24]. A control test was also performed containing inoculated broth supplemented with ethanol only at the same dilutions used in our experiments and inactive in culture me-

Table 1. Physical properties, preparation and spectral data of the compounds **(I-II)**.



Comp. No.	Y	R	Empirical Formula	mp (°C) *	Yield %	IR (cm^{-1})	^{1}H NMR δppm J = Hz
Ia	-	-H	$C_{13}H_{10}O_4N_2$	297[a]	44	3407, 1644, 1529, 1428, 1338	10.10 (s, 1H, NH), 9.59 (s, 1H, OH), 8.79 (d, 1H, C-6′ H, $J_{6',4'}$=2.68), 7.53-8.05 (m, 6H, C-2, C-3, C-4, C-5, C-6, C-4′ H), 7,09 (d, 1H, C-3′ H, $J_{3',4'}$=8.97)
Ib	-	$-NO_2$	$C_{13}H_9O_6N_3$	288[a]	62	3375, 3227, 1660, 1554, 1436, 1343	9.54 (s, 1H, OH), 8.96 (d, 1H, C-6′ H, J=2.66), 8.19-8.39 (dd, 4H, C-2, C-3, C-5, C-6, $J_{2,3}$=8.67, $J_{5,6}$=8.7), 7.93-7.97 (dd, 1H, C-4′ H, $J_{4',3'}$=8.95, $J_{4',6'}$=2.67), 7.07 (d, , 1H, C-3′ H, $J_{3',4'}$=8.97)
Ic	-	$-OCH_3$	$C_{14}H_{12}O_5N_2$	257[b]	37	3399, 3275, 2961-2861, 1641, 1494, 1430, 1340	9.44 (s, 1H, OH), 8.81 (d, 1H, C-6′ H, $J_{6',4'}$=2.76), 8.01-7.75 (m, 3H, C-2, C-6, C-4′ H), 7.02-7.15 (m, 3H, C-3, C-5, C-3′ H), 3.85 (s, 3H, CH_3)
Id	-	$-C(CH_3)_3$	$C_{17}H_{18}O_4N_2$	287[b]	34	3421, 3308, 2820-2910, 1645, 1526, 1433, 1339	9.15 (s, 1H, NH), 9.07 (s, 1H, OH), 7.93-7.62 (m, 4H, C-2, C-6, C-4′, C-6′ H), 7.55 (d, 2H, C-3, C-5 H, $J_{3,2}$= $J_{5,6}$ =8.30), 7,05 (d, 1H, C-3′ H $J_{3',4'}$=8.92), 1.62 (s, 9H, CH_3)
IIa	$-CH_2-$	-H	$C_{14}H_{12}O_4N_2$	260[b]	33	3355, 3294, 2850-2950, 1653, 1546, 1440, 1333	8.91 (d, 1H, C-6′ H, $J_{6',4'}$=2.52), 8.67 (s, 1H, OH), 7.85 (dd, 1H, C-4′ H, $J_{4',3'}$=8.92, $J_{4',6'}$= 2.74), 7.28-7.42 (m, 5H, C-2, C-3, C-4, C-5, C-6 H), 6.92 (d, 1H, C-3′ H, $J_{3',4'}$=8.91), 3.78 (s, 2H, CH_2)

Table 1. Continue.

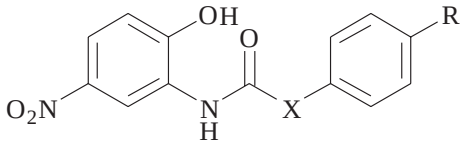
Comp. No.	Y	R	Empirical Formula	mp (°C) *	Yield %	IR (cm^{-1})	^{1}H NMR δppm J = Hz
IIb	$-CH_2-$	-Cl	$C1_4H_{11}O_4N_2$	249-252 (dec.) [b]	57	3395, 3294, 2900-2850, 1649,1540, 1433, 1343, 1080	11.54 (s, 1H, NH), 9.60 (s, 1H, OH), 8.94 (d, 1H, C-6′ H, $J_{6',4'}$=1.86), 7.89 (dd, 1H, C-4′ H, $J_{4',3'}$=8.87, $J_{4',6'}$= 2.48), 7.38 (s, 4H, C-2, C-3, C-5, C-6 H), 7.03 (d, 1H, C-3′ H, $J_{3',4'}$=8.93), 3.83 (s, 2H, CH_2)
IIc	$-CH_2-$	$-NO_2$	$C_{14}H_{11}O_6N_3$	259 [b]	38	3378, 3323, 2870-2950, 1658, 1517, 1431, 1347	11.65 (s, 1H, NH), 9.77 (s, 1H, OH), 8.93 (d, 1H, C-6′ H, $J_{6',4'}$=2.62), 8.20 (d, 2H, C-3, C-5 H, $J_{3,2}=J_{5,6}$=8.69), 7.90 (dd, 1H, C-4′ H, $J_{4',3'}$=8.94, $J_{4',6'}$= 2.83), 7.63 (d, 2H, C-2, C-6 H, $J_{2,3}=J_{6,5}$=8.62), 7.04 (d, J=8.95, 1H, C-3′ H, $J_{3',4'}$=8.93), 4.01 (s, 2H, CH_2)
IId	$-CH_2-$	-Br	$C_{14}H_{11}BrO_4N_2$	243 [b]	28	3394, 3265, 2899-2980, 1648, 1538, 1432, 1342, 1079	9.49 (s, 1H, OH), 8.94 (d, 1H, C-6′ H, $J_{6',4'}$=2.47), 7.85 (dd, 1H, C-4′ H, $J_{4',3'}$=8.98, $J_{4',6'}$= 2.80), 7.55 (d, 2H, C-3, C-5 H, $J_{3,2}=J_{5,6}$=8.25), 7.35 (d, 2H, C-2, C-6 H, $J_{2,3}=J_{6,5}$=8.23), 6.87 (d, 1H, C-3′ H, $J_{3',4'}$=9.01), 3.81 (s, 2H, CH_2)
IIe	$-CH_2-$	$-OC_3H_7$	$C_{17}H_{18}O_5N_2$	238 [b]	55	3354, 3323, 2877-2964, 1652, 1545, 1438, 1339	9.34 (s, 1H, OH), 8.94 (d, 1H, C-6′ H, $J_{6',4'}$=2.27), 7.85 (dd, 1H, C-4′ H, $J_{4',3'}$=8.95, $J_{4',6'}$= 2.66), 7.26 (d, 2H, C-2, C-6 H, $J_{2,3}=J_{6,5}$=8.29), 6.83-6,94(m, 3H, C-3, C-5, C-3′ H), 3.89 (t, J=6.47, 2H, $CH_3CH_2\underline{CH_2}O$), 3.72 (s, 2H, $\underline{CH_2}CONH$), 1.68-1.73 (m, 2H, $CH_3\underline{CH_2}CH_2O$), 0.96 (t, 3H, $\underline{CH_3}CH_2CH_2O$, J=7.32)

* Crystallization solvents; a : Acetone-Methanol, b: Methanol

dium in order to ensure that the solvent *per se* had no effect on bacterial growth. All the compounds were tested for their *in vitro* growth inhibitory activity against different bacteria and the yeast *Candida albicans RSKK 628*. The origins of the bacterial strains are *Staphylococcus aureus ATCC 6538, Streptococcus faecalis ATCC 10541* and *Bacillus subtilis ATCC 6033* as Gram-positive and *Klebsiella pneumoniae RSKK 256* and *Escherichia coli ATCC 10536* as Gram-negative bacteria. RSKK strains of the microorganisms used in this study were obtained from the culture collection of the Refik Saydam Hıfzısıhha Institute, Ankara, and maintained at the Microbiology Department of the Pharmacy Faculty of Ankara University.

As control drugs, ampicillin, amoxycillin, streptomycin, tetracycline, oxiconazole, and haloprogin were chosen. The observed data on the antimicrobial activity of the compounds and the control drugs are given in Table 2.

Table 2. The *in vitro* antimicrobial activity of the compounds **(I-II)** and the standard drugs (MIC in μg/mL.)



			Microorganisms*					
			Gram-positive			Gram-negative		Fungus
Comp. No:	X	R	Sa	Sf	Bs	Kp	Ec	Ca
Ia	–	-H	25	25	12.5	12.5	12.5	12.5
Ib	–	$-NO_2$	25	25	25	25	25	50
Ic	–	$-OCH_3$	25	25	25	25	25	12.5
Id	–	$-C(CH_3)_3$	12.5	12.5	12.5	25	25	12.5
IIa	$-CH_2-$	-H	25	25	12.5	25	25	12.5
IIb	$-CH_2-$	-Cl	25	25	25	25	25	12.5
IIc	$-CH_2-$	$-NO_2$	50	50	25	12.5	12.5	25
IId	$-CH_2-$	-Br	25	25	25	25	25	12.5
IIe	$-CH_2-$	$-OC_3H_7$	25	25	12.5	25	25	12.5
Ampicillin			1.56	1.56	1.56	25	12.5	-
Amoxycillin			1.56	1.56	1.56	12.5	3.12	-
Streptomycin			3.12	100	50	1.56	1.56	-
Tetracycline			1.56	1.56	1.56	3.12	3.12	-
Oxiconazole			-	-	-	-	-	6.2
Haloprogin			-	-	-	-	-	3.1

* Sa: Staphylococcus aureus Sf: Streptococcus faecalis Bs: Bacillus subtilis
Kp: Klebsiella pneumoniae Ec: Escherichia coli Ca: Candida albicans

Antibacterial and antifungal activity assay

Bacteria were cultured for 24 h at 37 $\pm$ 1 °C in Mueller-Hinton broth (Difco). The yeast *Candida albicans* was maintained in Sabouraud dextrose broth (Difco) after incubation for 24 h at 25 $\pm$ 1 °C. Testing was carried out in Mueller-Hinton broth and Sabouraud dextrose broth (Difco) at pH 7.4 and the 2-fold serial dilution technique was applied. The final inoculum size was 10^5 CFU/mL for the antibacterial assay and 10^4 CFU/mL for the antifungal assay. A set of tubes containing only inoculated broth was kept as a control. After incubation for 24 h at 37 $\pm$ 1 °C for the antibacterial assay and after incubation for 48 h at 25 $\pm$ 1 °C

for the antifungal assay, the last tube with no microorganism or yeast growth was recorded to represent the MIC expressed in μg/mL. Every experiment in the antibacterial and antifungal assays was replicated twice in order to define the MIC values.

Results and Discussion

The benzamides **Ia-d** and phenylacetamides **IIa-e** were synthesized in moderate yields by reacting 4-nitro-2-aminophenols with suitable carboxylic acid chlorides, obtained in turn by treating appropriate carboxylic acids with thionyl chloride. Their antimicrobial activities were determined by 2-fold serial dilution against 3 Gram-positive bacteria, 2 Gram-negative bacteria and the fungus *C. albicans*. Ampicillin, amoxycillin, streptomycin, and tetracycline were used as control drugs for antibacterial effect, and oxiconazole and haloprogin were used as standard drugs for antifungal effect to compare their antimicrobial activity.

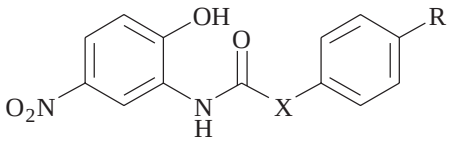
The antibacterial activity of the synthesized compounds and the control drugs shown in Table 2 indicated that the compounds **I-II** inhibited *in vitro* growth of a number of microorganisms, exhibiting MIC values of between 50 and 12.5 μg/mL. The derivative, N-(2-hydroxy-4-nitrophenyl)-p-(*t*-butylphenyl)benzamide, **Id,** was found to be more active than the others with a MIC value of 12.5 μg/mL against the bacteria *S. aureus* and *S. faecalis*. In addition, the compound **Id** significantly showed 3 dilutions better activity than its cyclic analogue, 2-(p-*t*-butylphenyl)-5-nitrobenzoxazole, **IIId**, shown in Table 3. The compounds **Ia, Id, IIa**, and **IIe** indicated notable activity, with a MIC value of 12.5 μg/mL against the bacterium *B. subtilis*. Moreover, all of the compounds were more active than streptomycin and less active than ampicillin, amoxycillin, and tetracycline as control drugs against *S. faecalis* and *B. subtilis*.

Furthermore, the compounds **I-II** showed significant activity against the bacteria *K. pneumonia* and *E. coli* with MIC values between 25 and12.5 μg/mL: 2 of them, **Ia** and **IIc,** were the most potent. All of the compounds exhibited lower antibacterial activities when compared to the standard drugs such as streptomycin and tetracycline against *K. pneumonia* and *E. coli*. Comparison of the synthesized compounds with their cyclic analogues, benzoxazole derivatives (**IIId, IVa-IVc**) showed these were one or 2 dilutions less active than their amide derivatives (**Id, IIa-IIc**) against *E. coli* (Table 3). In addition, the compounds **Id**, **IIa,** and **IIb** were more active than their cyclic derivatives against Gram-positive bacteria.

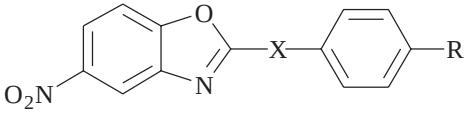
Moreover, Table 2 reveals that all of the synthesized compounds showed antifungal activity at a MIC value of 12.5 μg/mL against the fungus *C. albicans*, except for **Ib** and **IIc.** However, they exhibited one and 2 dilutions lower antifungal potencies than the standard drugs oxiconazole and haloprogin, respectively. Interestingly, most of the compounds, that were compared to their heterocyclic analogues given in Table 3 showed the same activity as their benzoxazole derivatives at 12.5 μg/mL, except for the compound **IIc,** against the same fungus**.**

In conclusion, as both amides and their cyclic analogues showed comparable activities, we may say that their pharmacophoric groups are similar and the possible metabolites of the suitable fused heterocyclics could be expected to give prolonged antimicrobial activity.

Table 3. Comparison of the antimicrobial activity of the synthesized benzamides and substitutedphenylacetamides **I-II** with their cyclic analogues **III-IV** (MIC μg/mL.)



I-II



III-IV

			Microorganisms*				
			Gram-positive		Gram-negative		Fungus
Comp. No:	X	R	Sa	*Sf*	*Kp*	*Ec*	*Ca*
Ia	–	-H	25	25	12.5	12.5	12.5
IIIa	–	-H	12.5	100	12.5	12.5	12.5
Id	–	$-C(CH_3)_3$	12.5	12.5	25	25	12.5
IIId	–	$-C(CH_3)_3$	100	100	100	100	12.5
IIa	$-CH_2-$	-H	25	25	25	25	12.5
IVa	$-CH_2-$	-H	50	50	12.5	50	12.5
IIb	$-CH_2-$	-Cl	25	25	25	25	12.5
IVb	$-CH_2-$	-Cl	50	50	12.5	50	12.5
IIc	$-CH_2-$	$-NO_2$	50	50	12.5	12.5	25
IVc	$-CH_2-$	$-NO_2$	50	50	12.5	50	12.5

* Sa: Staphylococcus aureus Sf: Streptococcus faecalis Kp: Klebsiella pneumoniae Ec: Escherichia coli Ca: Candida albicans

Acknowledgments

We would like to thank the Research Fund of Ankara University (Grant No. 96 03 00 04) for its financial support of this research.

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